

The claims have been amended to more particularly point out Applicants' invention. In particular, Claim 1 has been amended by reciting that at least one modification occurs in one or more genes which are different from the gene of interest. This is supported by the specification<sup>2</sup> which discloses the use of inorganic chemicals that generate multiple mutations in a plurality of genes.

Claims 1 and 15 have been amended to recite that the target cells are "isolated embryonic cells" as supported by the specification<sup>3</sup> which describes using the exemplary isolated embryonic stem cells, fertilized egg cells, and 2-cell embryos. Claims 1 and 15 have also been amended to recite that the agent is a "chemical" as supported by the specification.<sup>4</sup> Claim 15 has also been amended by reciting that the gene of interest is "in the isolated cells."

Claims 3 and 17 have been amended to further describe a step for generating an organism from the embryonic cells which have a modified gene of interest.

Claims 9, 13, 23 and 27 have been amended to provide antecedent basis for the term "embryonic cell."

Claims 13 and 27 have additionally been amended to recite that the embryonic stem cells are from "mouse" as supported by the specification.<sup>5</sup>

Claims 14 and 28 have been amended by deleting "ultraviolet light, X-ray radiation, and gamma-radiation" to avoid lack of antecedent basis.

The new claims have been added to more clearly describe Applicants' invention. Claims 29 and 32 have been added to recite that the target cells are "isolated embryonic cells selected from protocorm-like body cells, and callus cells" as supported by the specification.<sup>6</sup>

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<sup>2</sup> Specification, page 4, lines 11-17, page 6, lines 3-10, page 7, lines 14-21, paragraph bridging pages 12 and 13, and page 22, lines 16-29.

<sup>3</sup> Specification, page 19, lines 21-28; page 20; page 21; and page 22, lines 1-11.

<sup>4</sup> Specification, page 4, lines 11-17, page 6, lines 3-10, page 7, lines 14-21, paragraph bridging pages 12 and 13, and page 22, lines 16-29.

<sup>5</sup> Specification, paragraph bridging pages 20 and 21, Example 1, beginning on page 29 , Example 2 beginning on page 35, and Example 3 beginning on page 37.

<sup>6</sup> Specification, page 19, lines 25-28, page 27, lines 13-28, and page 28, lines 1-12.

Claims 30 and 33 find support in the specification<sup>7</sup> which discloses generating transgenic mice using ES cells. Claims 31 and 34 are supported by the specification<sup>8</sup> which discloses that the invention contemplates generating chimeric organisms.

Each of the amendments herein was made to expedite Applicants' business interests, without acquiescing to any of the Examiner's arguments, while expressly reserving the right to prosecute the un-amended (or similar) claims in another application. Applicants' amendments do not introduce new matter.

Claims 1-28 have been rejected on the following grounds:

1. Claims 3, 13, 17 and 27 stand rejected under 35 U.S.C. §112, first paragraph, for alleged lack of enablement;
2. Claims 3 and 17 were rejected under 35 U.S.C. §112, second paragraph for alleged indefiniteness;
3. Claims 1, 2, 4, 6, 7, 9-11, 13-16, 18, 20, 21, 23-25, 27, and 28 stand rejected under 35 U.S.C. §102(a) as being allegedly anticipated by Thomas *et al.*;
4. Claims 1, 2, 4, 6, 8, 9-11, and 13 were rejected under 35 U.S.C. §102(a) for alleged anticipation by Cohen-Tannoudji *et al.*;
5. Claims 1-7, 9-11, 14-21, 23-25 and 28 stand rejected under 35 U.S.C. §102(b) for alleged anticipation by Marker *et al.*;
6. Claims 1, 9, 12, 15, 23, and 26 were rejected under 35 U.S.C. §103(a) for alleged obviousness over Marker *et al.* and Schulte-Merker *et al.*; and
7. Claims 1, 6, 8, 15, 20, and 22 were rejected under 35 U.S.C. §103(a) for alleged obviousness over Marker *et al.* and Slamenova *et al.*.

Applicants believe that the present amendments and the following remarks traverse the Examiner's rejection of the claims. These remarks are presented in the same order as they appear above.

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<sup>7</sup> Specification, page 22, lines 3-11 and page 28, lines 3-12.

<sup>8</sup> Specification, page 22, lines 8-11, and page 28, lines 13-17.

### 1. Rejection of Claims 3, 13, 17 and 27 Under 35 U.S.C. §112, First Paragraph

Claims 3, 13, 17 and 27 stand rejected under 35 U.S.C. §112, first paragraph, for alleged lack of enablement<sup>9</sup> on the basis that "it is a huge leap to go from manipulation of a cell to the generation of a non-human animal,"<sup>10</sup> and "the specification fails to enable methods involving the manipulation of embryonic stem cells for any species other than for mice."<sup>11</sup> Applicants respectfully traverse because generic methods (in combination with the teachings of the specification) may be used to generate the recited organisms.

Referring specifically to rejected Claims 3 and 17, the Examiner concluded that "it is a huge leap to go from manipulation of a cell to the generation of a non-human animal," on the basis of three observations. First, the Examiner found that "it is unknown how a cell other than an embryonic stem cell would ever be capable of germline contribution since it is well known that only embryonic stem cells can contribute to germline formation of tissues and the whole animal."<sup>12</sup> The Examiner is asked to take note of the fact that the claims do **not** require that the modified target cells be capable of **germline** contribution. Indeed, the specification contradicts the Examiner's implication for such a requirement when it states that:

"the invention also expressly contemplates chimeric organisms (*i.e.*, organisms which contain a transgene in only *somatic* cells) . . . [and] [t]he regenerated animals, . . . whether they contain a modified gene of interest in a somatic and/or germline cell, may be used to determine the function of the gene of interest."<sup>13</sup>

In other words, the specification confirms the plain language of the claims by unambiguously stating that the transgenic organisms may contain the recited genetic modifications in either somatic cells alone, germline cells alone, or both somatic and germline cells.<sup>14</sup> Accordingly,

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<sup>9</sup> Office Action, page 3.

<sup>10</sup> Office Action, page 4, final paragraph.

<sup>11</sup> Office Action, page 5, final paragraph.

<sup>12</sup> Office Action, page 4, final paragraph.

<sup>13</sup> Emphasis added, specification, page 28, lines 15-21.

<sup>14</sup> Applicants note that new Claims 29 and 32 more particularly point out that Applicants' invention includes cells which are capable of somatic cell contribution **and/or** germline contribution, and new Claims 31 and 34 specifically recite that the resulting organisms

the Examiner's first observation is premised on an erroneous narrowing of the scope of the claims to only those cells capable of germline contribution. Because of this error, and because the recited "embryonic stem cells, fertilized egg cells, and cells of 2-cell embryos"<sup>15</sup> are known to contribute to the formation of somatic cells in multicellular organisms, the Examiner's first observation is misguided.<sup>16</sup>

Second, the Examiner observed that "it is unclear what Applicant intends to be encompassed within the 'manipulation' of a cell."<sup>17</sup> The Examiner is respectfully reminded that:

"[p]atents . . . are written to enable those skilled in the art to practice the invention."<sup>18</sup>

It is submitted that it is within the ordinary skill in the art to review the prior art to determine and practice generic steps for "manipulating" the target embryonic stem cells, fertilized egg cells, and 2-cell embryos in order to generate multicellular organisms.<sup>19</sup> One of ordinary skill in the art is also cognizant that these steps depend on the target cells used to generate the organisms. This is in fact demonstrated by the Examiner's understanding<sup>20</sup> that with respect

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are **chimeric**. Applicants also note that the Examiner's own reference (*Mullins et al.*) concedes enablement of chimeric animals since it states that "chimeric animals have been generated from several species including the pig." Office Action, page 5, final paragraph.

<sup>15</sup> Specification, page 19, lines 23-28.

<sup>16</sup> Applicants' comments are equally applicable to the "protocorm-like body cells, and callus cells" recited in new Claims 29 and 32 because these embryonic cells are known to contribute to the formation of multicellular plants.

<sup>17</sup> Office Action, page 4, final paragraph.

<sup>18</sup> (Emphasis added) *W.L. Gore & Assoc., Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1556, 220 USPQ 303, 315 (Fed. Cir. 1983), cert. denied, 105 S. Ct. 172 (1984).

<sup>19</sup> Applicants' comments are equally applicable to the "protocorm-like body cells, and callus cells" recited in new Claims 29 and 32 because it is within the artisan's skill to review the prior art to practice generic steps to generate multicellular plants from these cells.

<sup>20</sup> Applicants argument does not suggest or concede that the Examiner is a person with ordinary skill in the art.

to target embryonic stem cells, the steps of "manipulating" these cells to generate organisms include "introducing an embryonic stem cell (having a modified gene of interest) into an embryo, transplanting the embryo into a recipient non-human animal, allowing the embryo to develop to term . . ."<sup>21</sup> Since practicing the steps to generate a multicellular organism from an embryonic cell are within the skill in the art, this ground of the Examiner's rejection is unsound.

Third, the Examiner also remarked that the generation of transgenic organisms "involves essential steps" and "it is strongly suggested that the claims be amended to include the essential steps."<sup>22</sup> Applicants respectfully suggest that the Examiner's suggestion to amend Claims 3 and 17 is unduly limiting.

First, while the Examiner's suggested steps may apply to manipulating embryonic stem cells, they do not apply to manipulating **other embryonic cells** (*i.e.*, "fertilized egg cells, and cells of 2-cell embryos"<sup>23</sup>) which are within the scope of Claims 3 and 17. For example, 2-cell embryos need not be introduced into an embryo (they **are** an embryo), but rather introduced directly into a uterus.

Second, the steps referred to by the Examiner are not essential, but merely preferred.<sup>24</sup> The law is clear that "features that are merely preferred are not critical."<sup>25</sup> Thus, with respect to embryonic stem cells,<sup>26</sup> the specification discloses that these cells may be manipulated in alternative ways to generate multicellular animals, and that some of the steps suggested by the Examiner represent only preferred (and therefore, **not critical**) steps. For example, the

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<sup>21</sup> Office Action, sentence bridging pages 4 and 5.

<sup>22</sup> Office Action, paragraph bridging pages 4 and 5.

<sup>23</sup> Specification, page 19, lines 23-28.

<sup>24</sup> Applicants note that new Claims 30 and 33 recite steps encompassed by "manipulating" the modified target cells. Because these claims conform to the Examiner's suggestion, they are enabled.

<sup>25</sup> *In re Goffe*, 542 F.2d 564, 191 USPQ 429 (CCPA 1976), citing *In re Armbruster*, 512 F.2d 676, 185 USPQ 152 (CCPA 1975) and *In re Geerdes*, 491 F.2d 1260, 180 USPQ 789 (CCPA 1974); MPEP 2164.08(c).

<sup>26</sup> The Examiner's suggested amendment includes the limitation of using embryonic stem cells as target cells for introducing the modifications to the gene of interest.

specification says that embryonic stem cells may be injected into blastocysts<sup>27</sup> or alternatively sandwiched between two eight-cell embryos.<sup>28</sup> Because the steps suggested by the Examiner represent one of the specification's alternative (not critical) steps, these steps need not be included in the claims to satisfy enablement.

Third, the Examiner's suggested list of additional steps improperly narrows the scope of the claims by including the step of "breeding the transgenic non-human animal to produce a transgenic non-human animal whose genome comprises the modified gene of interest in *both alleles*."<sup>29</sup> This narrow scope is contradicted by the specification. The specification clearly explains that the methods of the invention are **not** limited to generating organisms which have in modification in **both alleles** of a gene. It says:

"The regenerated animals, whether heterozygous or homozygous for a modification in a gene of interest, and whether they contain a modified gene of interest in a somatic and/or germline cell, may be used to determine the function of the gene of interest."<sup>30</sup>

Thus, organisms which are heterozygous or homozygous for the modification in the gene of interest are included within the scope of the claimed invention. Accordingly, the step of "breeding" to generate homozygous organisms need not be included in the claims.

Notwithstanding the Examiner's error in construing the scope of the claims, and without acquiescing to any of the Examiner's arguments, but merely to expedite Applicants' business interests without waiving the right to prosecute the originally-filed (or similar) claims in another application, Applicants have amended Claims 1 and 15.

In view of the above arguments and amendments, it is respectfully requested that the rejection of Claims 3, 13, 17 and 27 under 35 U.S.C. §112, first paragraph, be withdrawn.

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<sup>27</sup> Specification, page 21, lines 6-12, and page 28, lines 6-8.

<sup>28</sup> Specification, page 28, lines 8-11.

<sup>29</sup> (Emphasis added) Office Action, page 5 first paragraph.

<sup>30</sup> Specification, page 28, lines 18-21.

## 2. Rejection of Claims 3 and 17 Under 35 U.S.C. §112, Second Paragraph

Claims 3 and 17 were rejected under 35 U.S.C. §112, second paragraph for alleged indefiniteness of the term "manipulating."<sup>31</sup> Applicants respectfully must disagree. The analysis under §112, second paragraph,

"requires a determination whether those skilled in the art would understand what is claimed."<sup>32</sup> "It is entirely proper to use the specification to interpret what the patentee meant by a word or phrase in the claim."<sup>33</sup>

Applicants assert that the meaning of the term "manipulating" is understood by one of ordinary skill in the art as discussed below.

The Examiner first argued that the claims are "incomplete" because the "only step of the method towards generation of a non-human animal is the 'manipulation' of cells having a modified gene of interest."<sup>34</sup> However, one of ordinary skill in the art understands from the specification that the nature of "manipulating" depends on the type of modified embryonic cell. Thus, with respect to fertilized egg cells of a mammal, the specification teaches that:

"transgenic mammals are generated by implanting the treated fertilized egg cell into the uterus of a pseudopregnant female and allowing the cell to develop into an animal. This method has been successful in producing transgenic mice, sheep, pigs, rabbits and cattle [Jaenisch (1988) *supra*; Hammer *et al.*, (1986) J. Animal Sci.:63:269; Hammer *et al.*, (1985) Nature 315:680-683; Wagner *et al.*, (1984) Theriogenology 21:29]."<sup>35</sup>

In addition, with respect to fertilized egg cells from fish, (*e.g.*, zebrafish), it is taught that:

"transgenic zebrafish may be generated by allowing the fertilized egg cell to develop without the need for attention from its parents."<sup>36</sup>

Referring to embryonic stem cells, the specification explains that:

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<sup>31</sup> Office Action, page 6.

<sup>32</sup> *Amgen, Inc. v. Chugai Pharm. Co.*, 927 F.2d 1200, 1217 , 18 USPQ2d 1016 (Fed. Cir. 1991).

<sup>33</sup> *E.I. du Pont de Nemours & Co. v. Phillips Petroleum Co.*, 849 F.2d 1430, 1433 (Fed. Cir. 1988); 37 C.F.R. §1.75(d)(1).

<sup>34</sup> (Emphasis added) Office Action, page 7, first paragraph.

<sup>35</sup> Specification, page 27, lines 13-19.

<sup>36</sup> Specification, page 27, lines 20-23.

"where the cell treated with the nucleic acid sequence-modifying agent is a ES cell, multicellular organisms may be generated by introducing the modified ES cell back into the embryonic environment for expression and subsequent transmission to progeny animals. The most commonly used method is the injection of several ES cells into the blastocoel cavity of intact blastocysts [Bradley *et al.*, (1984) *Nature* 309:225-256]. Alternatively, a clump of ES cells may be sandwiched between two eight-cell embryos [Bradley *et al.*, (1987) in "Teratocarcinomas and Embryonic Stem Cells: A Practical Approach," Ed. Robertson E.J. (IRL, Oxford, U.K.), pp. 113-151; Nagy *et al.*, (1990) *Development* 110:815-821]."<sup>37</sup>

Thus, one of ordinary skill in the art who reads the above disclosure is apprised that the term "manipulating" the modified target cells to generate organisms refers to the **totality of step(s)** which are sufficient to generate the organism, and that the precise nature of these generic steps depends on the type of target cell. Because the scope of the claims is clear to a hypothetical person possessing the ordinary level of skill in the pertinent art<sup>38</sup> in view of the specification, the term "manipulating" is definite.

The Examiner next argued that the term "manipulating" is indefinite because "the generation of a transgenic non-human animal generally requires *essential steps* . . . "<sup>39</sup>. However, as discussed above, these additional steps are inappropriate to the originally-filed claims 3 and 17 because the allegedly "essential steps" refer to manipulating embryonic stem cells, while rejected Claims 3 and 17 are **not** limited to embryonic stem cells. Rather, Claims 3 and 17 encompass cells other than embryonic stem cells, including "fertilized egg cells, and cells of 2-cell embryos."<sup>40</sup>

Moreover, The MPEP directs the Examiner that a rejection under 35 U.S.C. §112, first paragraph is warranted only if:

"[a] feature which is taught as critical in a specification . . . is not recited in the claims."<sup>41</sup>

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<sup>37</sup> Specification, page 28, lines 3-11.

<sup>38</sup> MPEP §2171.

<sup>39</sup> Office Action, page 7, first paragraph.

<sup>40</sup> Specification, page 19, lines 23-28.

<sup>41</sup> MPEP 2164.08(c), citing *In re Mayhew*, 527 F.2d 1229, 188 USPQ 356 (CCPA 1976).

However, nowhere does the specification even suggest that the allegedly "essential steps" are critical for generating the recited organisms. To the contrary, the specification discloses that generating the recited organisms from manipulated fertilized egg cells (which are within the scope of Claims 3 and 17) can proceed **without** resort to the allegedly "essential steps" listed by the Examiner (*i.e.*, by implanting the treated mammalian fertilized egg cell into the uterus of a pseudopregnant female and allowing the cell to develop into an animal<sup>42</sup> and by allowing the fertilized egg cell from a fish to develop without the need for attention from its parents<sup>43</sup>). Accordingly, the alleged "essential steps" are **not** a critical feature, and their omission from Claims 3 and 17 does not result in indefiniteness.<sup>44</sup>

For the above reasons, it is submitted that the term "manipulating" is definite. Nonetheless, to expedite Applicants' business interests, without acquiescing to any of the Examiner's arguments, while expressly reserving the right to prosecute the un-amended (or similar) claims in another application, Applicants have amended Claims 3 and 17 to further describe the step for generating an organism from the embryonic cells which have a modified gene of interest. Accordingly, it is respectfully requested that the rejection of Claims 3 and 17 be withdrawn.

3. **Rejection of Claims 1, 2, 4, 6, 7, 9-11, 13-16, 18, 20, 21, 23-25, 27, and 28 Under 35 U.S.C. §102(a) Over Thomas *et al.***

Claims 1, 2, 4, 6, 7, 9-11, 13-16, 18, 20, 21, 23-25, 27, and 28 stand rejected under 35 U.S.C. §102(a) as being allegedly anticipated by Thomas *et al.*<sup>45</sup> Applicants respectfully disagree.

Thomas *et al.* is not prior art under §102(a) since it was not invented by other than Applicants. Applicants provide an *In re Katz* Declaration executed by co-inventor Dr.

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<sup>42</sup> Specification, page 27, lines 13-19.

<sup>43</sup> Specification, page 27, lines 20-23.

<sup>44</sup> Applicants note that new Claims 30 and 33, which recite embryonic stem cells, also include steps for manipulating these cells to generate organisms, thus obviating a rejection of these claims under 35 U.S.C. §112, second paragraph.

<sup>45</sup> Office Action, page 7, last paragraph.

Magnuson stating that co-author Christian LaMantia worked under the supervision and direction of co-inventors Terry Magnuson and James W. Thomas. Accordingly, Applicants respectfully request that this rejection be withdrawn.

**4. Rejection of Claims 1, 2, 4, 6, 8, 9-11, and 13 Under 35 U.S.C. §102(a) Over Cohen-Tannoudji et al.**

Claims 1, 2, 4, 6, 8, 9-11, and 13 were rejected under 35 U.S.C. §102(a) for alleged anticipation by Cohen-Tannoudji *et al.*<sup>46</sup> Applicants respectfully disagree since this reference does not disclose all the limitations of the amended claims. The law is clear that:

"Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration."<sup>47</sup> "[A]bsence from the reference of any claimed element negates anticipation."<sup>48</sup>

However, Cohen-Tannoudji *et al.* does not disclose using a "chemical agent" which is capable of producing at least one modification in a gene of interest as well as in "one or more genes" as recited in step a)ii) of the rejected claims. Cohen-Tannoudji *et al.* discloses a method for enhancing homologous recombination of the ezrin cDNA into the villin gene of embryonic stem cells. In this method, the I-SceI restriction site is first introduced into the cells' endogenous villin locus. This is followed by co-electroporation into these cells of an expression vector which expresses the I-SceI restriction enzyme and a replacement construct which contains the ezrin DNA. The expressed I-SceI restriction enzyme creates a double-strand break in the villin gene at the introduced I-SceI restriction site and results in a high frequency of targeting of the ezrin cDNA into the villin locus.

Importantly, Cohen-Tannoudji *et al.* does not disclose using a "chemical agent" as recited by the rejected claims.

Moreover, the method of Cohen-Tannoudji *et al.* relies on the introduction of a single double-strand break in a **single** gene (*i.e.*, the villin locus) to achieve precise targeting of the

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<sup>46</sup> Office Action, page 9, last full paragraph.

<sup>47</sup> *W.L. Gore & Assoc., Inc v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303, 313 (Fed. Cir. 1983), cert. denied, 105 S. Ct. 172 (1984), citing *Soundscriber Corp. v. U.S.*, 360 F.2d 954, 960, 148 USPQ 298, 301, adopted, 149 USPQ 640 (Ct. Cl. 1966).

<sup>48</sup> *Rowe v. Dror*, 42 USPQ2d 1550, 1553 (Fed. Cir. 1997), citing *Kloster Speedsteel AB v. Crucible, Inc.*, 793 F.2d 1565, 1571, 230 USPQ 81, 84 (Fed. Cir. 1986).

ezrin cDNA into the villin locus. This is in contrast to the claimed invention which recites using an agent that is capable of producing modifications in a gene of interest **and** in one or more other genes.

Because Cohen-Tannoudji *et al.* does not identically disclose all the elements of the rejected claims, it cannot anticipate these claims. Accordingly, the Examiner is respectfully requested to withdraw the rejection of Claims 1, 2, 4, 6, 8, 9-11, and 13 under 35 U.S.C. §102(a) over Cohen-Tannoudji *et al.*

**5. Rejection of Claims 1-7, 9-11, 14-21, 23-25 and 28 Under 35 U.S.C. §102(b) Over Marker et al.**

Claims 1-7, 9-11, 14-21, 23-25 and 28 stand rejected under 35 U.S.C. §102(b) for alleged anticipation by Marker *et al.*<sup>49</sup> Applicants respectfully traverse since Marker *et al.* fails to disclose several limitations of the claims.

In the first instance, Marker does not disclose the limitation of "isolated embryonic cells" as recited in step a)i) of each of the amended claims. Marker *et al.* discloses treating mice with chemicals or radiation to induce mutations in the *short ear* locus. Viable mice containing 24 mutations at the *short ear* locus (which encodes the Bmp5 protein) were recovered and used to generate animals which are homozygous for mutations in the *short ear* alleles.<sup>50</sup> The effects of these mutations on the animals were used to investigate the function of the TGF $\beta$  superfamily, of which the Bmp5 protein is a member. Importantly, the method of Marker *et al.* is distinguished from the claimed methods in that Marker *et al.*'s mutations in the *short ear* locus were induced by treatment of **whole animals**, not of **isolated embryonic cells**.

Marker *et al.* also fails to disclose "isolating" the modified cells as recited in step c) of Claims 1-7, 9-11, 15-21, 23-25 and 28. It is important to note that Marker *et al.* discloses generating mice which contain mutations in the *short ear* locus, and that the mutations in this locus are determined by using tissue (not isolated cells) from these mice as a source of

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<sup>49</sup> Office Action, page 10, final full paragraph.

<sup>50</sup> Marker *et al.*, page 436, first column.

DNA.<sup>51</sup> In other words, Marker *et al.* did not **isolate cells** which contain modifications in the *short ear* locus, but rather determined modifications to this gene in **whole tissue**.

Moreover, Marker *et al.* does not disclose "producing an allelic series of modifications in said gene of interest in the isolated cells" as recited in step c) of Claims 15-21, 23-25 and 28. Marker *et al.* discloses treating mice with chemicals and radiation to induce mutations in the *short ear* locus, outcrossing progeny animals with altered size and shape of the external ear to a *dilute* strain, and intercrossing the long eared, dark-coated progeny animals. However, none of the steps used by Marker *et al.* includes producing an allelic series of modifications in the *short ear* locus in **isolated cells**. Rather, Marker *et al.*'s modifications to the *short ear* locus reside solely in **whole animals or whole tissue**.

The absence of several limitations from Marker *et al.* negates anticipation of the claims by this reference. Accordingly, Applicants respectfully request that the rejection of Claims 1-7, 9-11, 14-21, 23-25 and 28 under 35 U.S.C. §102(b) be withdrawn.

6. **Rejection of Claims 1, 9, 12, 15, 23, and 26 Under 35 U.S.C. §103(a) Over Marker *et al.* in view of Schulte-Merker *et al.***

Claims 1, 9, 12, 15, 23, and 26 were rejected under 35 U.S.C. §103(a) for alleged obviousness over Marker *et al.* in view of Schulte-Merker *et al.*<sup>52</sup> Applicants respectfully traverse because a *prima facie* case of obviousness is not established.

A *prima facie* case of obviousness requires the Examiner to cite to a combination of references which (a) suggests or motivates one of skill in the art to modify their teachings to yield the claimed invention, (b) discloses the elements of the claimed invention, **and** (c) provides a reasonable expectation of success should the claimed invention be carried out. Failure to establish **any** one of these requirements precludes a finding of a *prima facie* case of obviousness and, without more, entitles Applicants to allowance of the claims in issue.<sup>53</sup>

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<sup>51</sup> Marker *et al.*, Materials and Methods, beginning on page 435; in particular, see, first full paragraph on page 436.

<sup>52</sup> Office Action, page 12, last paragraph.

<sup>53</sup> See, e.g., *Northern Telecom Inc. v. Datapoint Corp.*, 15 USPQ2d 1321, 1323 (Fed. Cir. 1990); *In re Dow Chemical Co.*, 837 F.2d 469, 5 USPQ2d 1529 (Fed. Cir. 1988).

Applicants urge that the Examiner has failed to establish not one, but **all three** requirements as discussed below.

**A. The Combined References Do Not Provide Motivation To Practice The Claimed Methods - *In Re Rouffet***

The Federal Circuit<sup>54</sup> held that it is improper for the Patent Office to consider references collectively before establishing the threshold requirement that a person skilled in the art would be **motivated** to combine these references in the first place. Motivation to combine or modify the references requires that these references suggest the desirability of the **claimed invention**.<sup>55</sup> The Examiner's argument in support of motivation falls short of the legal test as explained below.

The Examiner stated that "[o]ne of ordinary skill in the art would have been motivated to perform mutagenesis experiments in the zebrafish system because the zebrafish combines advantages of both amphibian and mammalian systems, and particularly since the zebrafish exhibit some level of conservation in many mammalian genes such as the mouse T gene."<sup>56</sup>

However, it is notable that the Examiner's "motivation" argument refers specifically to using zebrafish in the invention's assays. However, rejected Claims 1 and 15 are not limited to cells from zebrafish, but to target embryonic cells. Thus, any alleged motivation with respect to using zebrafish does not fulfill the necessary motivation to use target embryonic cells from sources which are other than zebrafish and which fall within the scope of Claims 1 and 15.

As to rejected Claims 12 and 26 which recite zebrafish, the Examiner also took the position that "Schulte-Merker *et al.* point out that zebrafish combine the advantages of the amphibian and the mammalian system, and that, as in mice, genetic analysis in zebrafish is

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<sup>54</sup> *In re Rouffet*, 47 USPQ2d 1453 (Fed. Cir. (1998)).

<sup>55</sup> *In re Fine*, 837 F.2d 1071, 1074, 5 USPQ2d 1596, 1598-99 (Fed. Cir. 1988) and *In re Jones*, 21 USPQ2d 1941, 1943 (Fed. Cir. 1992).

<sup>56</sup> Office Action, page 14, first full paragraph.

*possible.*<sup>57</sup> However, motivation is lacking because obviousness requires more than knowledge of the individual steps in a claimed combination. The law is that:

"That which is within the capabilities of one skilled in the art is not synonymous with obviousness."<sup>58</sup>

At best, the Examiner's comment regarding obviousness amounts to an assertion that one of ordinary skill in the relevant art would have been able to arrive at Applicants' claimed **combination** in Claims 12 and 26 because he had the necessary skills to carry out some of the requisite **individual** process steps. This is an inappropriate standard for obviousness; the prior art must fairly suggest combining the various individual steps in the various cited references.

Because this element has not been established with respect to any of the rejected claims, a *prima facie* case of obviousness must fail.

#### B. The References Do Not Teach All The Elements Of The Rejected Claims

It is axiomatic for establishing a *prima facie* case of obviousness that "all the claim limitations must be taught or suggested by the prior art."<sup>59</sup> This has not been established since the references do not teach all the claims' limitations.

The Examiner conceded that the primary Marker *et al.* reference "differ[s] from the claimed invention in that they do not specifically teach mutagenesis in cells of the zebrafish."<sup>60</sup> This is not the only problem with Marker *et al.*; Marker *et al.* does not disclose the limitation that the target embryonic cells are "isolated" as recited in step a)i) in each of rejected Claims 1, 9, 12, 15, 23 and 26. As discussed above,<sup>61</sup> Marker *et al.* discloses using

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<sup>57</sup> (Emphasis added) Office Action, page 13, second paragraph.

<sup>58</sup> *Ex parte Levengood*, 28 USPQ2d 1300, 1302 (Pat. Bd. Appeals & Interf. 1993), citing *Ex parte Gerlach*, 212 USPQ 471 (Pat. Bd. Appeals & Interf.. 1980). See, also fn. 16 of *Panduit Corp. v. Dennison Mfg. Co.*, 774 F.2d 1082, 1092, 227 USPQ 337, 343 (Fed. Cir. 1985).

<sup>59</sup> MPEP 2143.03, citing *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974).

<sup>60</sup> Office Action, page 13, second paragraph.

<sup>61</sup> Item 5, beginning on page 18 of this communication.

whole animals, not "isolated embryonic cells." Schulte-Merker *et al.* does not overcome the deficiency in Marker *et al.*'s disclosure. Schulte-Merker *et al.* discloses the expression pattern of the zebrafish *Zf-T* gene (which is a homologue of the mouse *T* gene) and the effect of modifying expression of the *Zf-T* gene, using antibodies, on mesoderm formation. This reference concludes that the molecular mechanisms that regulate mesoderm formation in fish and mammals are very similar.<sup>62</sup> Thus, Schulte-Merker *et al.* nowhere refers to using "isolated embryonic cells" as targets for treatment, nor using a "chemical agent" to modify a gene of interest in these cells.

Moreover, as discussed above with respect to Claims 1 and 15,<sup>63</sup> Marker *et al.* fails to disclose "isolating" the modified cells as recited in step c) of rejected Claims 1, 9, 12, 15, 23 and 26. Nor does Schulte-Merker *et al.* provide this missing element since this reference is concerned with modifying expressed **protein** using an antibody (rather than modifying a **gene** of interest), and with analyzing the effects of such expression in the **whole embryo** (rather than in **isolated embryonic cells**).

In addition, for the reasons discussed above,<sup>64</sup> Marker *et al.* does not disclose "producing an allelic series of modifications in said gene of interest in the isolated cells" as recited in step c) of rejected Claims 15, 23 and 26. Schulte-Merker *et al.* is also deficient because it does not relate to modifying a gene of interest<sup>65</sup> or to isolated cells,<sup>66</sup> let alone producing an allelic series of such a modified gene in isolated cells.

Because all the elements of the rejected claims are not taught by the combined teachings of the prior art, a *prima facie* case of obviousness cannot stand.

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<sup>62</sup> Schulte-Merker *et al.* page 1022, first column, second full paragraph.

<sup>63</sup> Item 5, beginning on page 15 of this communication.

<sup>64</sup> *Id.*

<sup>65</sup> Schulte-Merker *et al.* instead relates to modifying expressed **protein**.

<sup>66</sup> Schulte-Merker *et al.* instead relates to **whole embryos**.

### C. A Reasonable Expectation of Success In Practicing The Claimed Methods Is Not Established

The Examiner argued that "it would have been obvious for one of ordinary skill in the art, at the time the claimed invention was made, to modify the mutagenesis methods of Marker *et al.* by substituting the zebrafish system for the mouse system with a reasonable expectation of success."<sup>67</sup> This statement suffers from two deficiencies.

The Examiner has failed to explain (as she must) why "substituting the zebrafish system for the mouse system" would have met with a reasonable expectation of success. In essence, the Examiner seems to be saying that it may have been obvious to try to use "isolated" target embryonic cells instead of whole animals, and to try using zebrafish cells instead of mouse to generate genetic modifications in the target cells. However, the law is clear that "obvious to try" is not the proper standard. Indeed, the "obvious to try" argument has been thoroughly discredited.<sup>68</sup>

Because the Examiner uses an improper legal standard to infer a "reasonable expectation of success," and because she has not demonstrated (as she must) that the reasonable expectation of success is founded in the prior art rather than in Applicants' disclosure,<sup>69</sup> the third prong of a *prima facie* case of obviousness is defective.

Because, not one, but each of the three elements of a *prima facie* case of obviousness is lacking, a *prima facie* case of obviousness cannot be established. It is therefore respectfully requested that the rejection of Claims 1, 9, 12, 15, 23 and 26 under 35 U.S.C. §103(a) for alleged obviousness over Marker *et al.* in view of Schulte-Merker *et al.* be withdrawn.

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<sup>67</sup> Office Action, page 14, first full paragraph.

<sup>68</sup> "[T]his court and its predecessors have repeatedly emphasized that "obvious to try" is not the standard under Section 103." *See In re O'Farrell*, 7 USPQ2d 1673, at 1680-1681 (Fed. Cir. 1988).

<sup>69</sup> *In re Dow Chemical Co.*, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988) as cited in *In re Vaeck*, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991).

7. **Rejection of Claims 1, 6, 8, 15, 20, and 22 Under 35 U.S.C. §103(a) Over Marker et al. in view of Slamenova et al.**

Claims 1, 6, 8, 15, 20, and 22 were rejected under 35 U.S.C. §103(a) for alleged obviousness over Marker *et al.* in view of Slamenova *et al.*<sup>70</sup> Applicants respectfully disagree since the Examiner has not established any of the elements of a *prima facie* case of obviousness.

**A. There Is No Motivation To Practice The Claimed Methods**

The Examiner's singular argument in support of a motivation to combine Marker *et al.* and Slamenova *et al.* is that "[o]ne of ordinary skill in the art would have been motivated to use MNNG to induce DNA breaks for study of DNA rejoining in mammalian cells as undertaken by Slamenova *et al.*, for example."<sup>71</sup>

This argument is conclusory and unsupported by any reasoning. The Examiner is respectfully reminded that an

" . . . examiner must show *reasons* that the skilled artisan, confronted with the same problems as the inventor and with no knowledge of the claimed invention, would select the elements from the cited prior art references for combination in the manner claimed."<sup>72</sup>

While the Examiner recognized the requirement for motivation by the prior art, she has not provided any **reasons** why such motivation exists. This deficiency alone negates a *prima facie* case of obviousness.

**B. The Elements Are Not Disclosed**

As discussed above in connection with Claims 1 and 15,<sup>73</sup> Marker *et al.* fails to disclose using "isolated embryonic cells" as recited in step a(i). Slamenova *et al.* also does not disclose this limitation because it refers to using only human cells and hamster cells.

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<sup>70</sup> Office Action, page 15, first full paragraph.

<sup>71</sup> Office Action, page 16., first full paragraph.

<sup>72</sup> (Emphasis added) *In re Rouffet*, 47 USPQ2d 1453 (Fed. Cir. (1998)).

<sup>73</sup> Item 5, beginning on page 18 of this communication.

Furthermore, Marker *et al.* does not disclose "isolating" the modified cells as recited in step c) of rejected Claims 1, 6, 8, 15, 20 and 22. This limitation is also absent from Slamenova *et al.* Slamenova *et al.* discloses exposure of human cells and hamster cells to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and evaluation by different techniques of the level of single strand breaks of DNA in these cells. Importantly, Slamenova *et al.*'s treatment of cells with MNNG results in a mixture of cells, some containing single strand breaks, while others are devoid of such breaks. Slamenova *et al.* evaluates this **mixture** of cells for single strand breaks, without resort to **isolating** only those cells which contain modifications. Thus, the combined references do not teach "isolating" the modified cells.

In addition, for the same reasons discussed *supra* with respect to Claim 15,<sup>74</sup> Marker *et al.* does not disclose "producing an allelic series of modifications in said gene of interest in the isolated cells" as recited in step c) of rejected Claims 15, 20 and 22. Slamenova *et al.* does not bridge the gap in Marker *et al.*'s deficiency because this reference is related to evaluating single strand breaks in a **mixture of cells**, rather than producing an allelic series of modified gene in **isolated cells**.

Moreover, with regards to rejected Claims 6 and 20, the references do not disclose modifications which include a mutation and mismatch. Rather, Slamenova *et al.* is concerned only with single strand breaks.

As to rejected Claims 8 and 22 which recite the limitation that the strand break is a "double-strand" break, the references do not even allude to this limitation. The only type of strand break disclosed by the cited references is a single strand break.<sup>75</sup>

Because the combined teachings of the references do not add up to the individual limitations recited in the rejected claims, a *prima facie* case of obviousness is not sustainable.

### C. A Reasonable Expectation Of Success Is Not Supported

In addressing this requirement of a *prima facie* case of obviousness, the Examiner asserted that "it would have been obvious for one of ordinary skill in the art, at the time the invention was made to modify their mutagenesis experiments by using the mutagen, MNNG,

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<sup>74</sup> *Id.*

<sup>75</sup> Slamenova *et al.*, page 247, first paragraph.

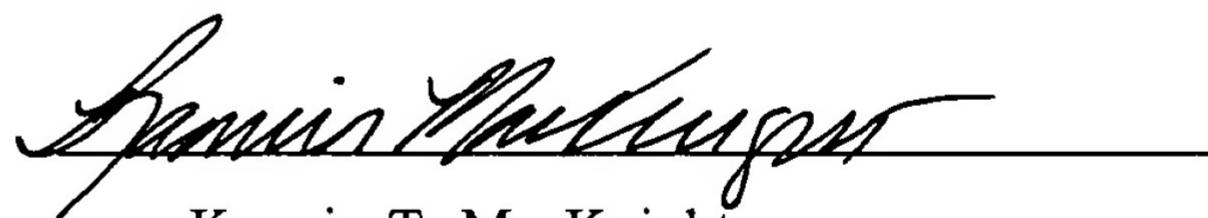
for induction of DNA breaks with a reasonable expectation of success.<sup>76</sup> This assertion is unsupported, and at best highly speculative. The Examiner does not explain why such alleged success is reasonably expected. Indeed, it is Applicants' position that such an expectation cannot logically be made when the references fail to even disclose the elements which are necessary for the combination of steps in the rejected methods. This requirement of a *prima facie* case is thus not met.

Since the Examiner has failed to meet the requirements of a *prima facie* case of obviousness, Applicants respectfully request withdrawal of the rejection of Claims 1, 6, 8, 15, 20, and 22 under 35 U.S.C. §103(a) over Marker *et al.* in view of Slamenova *et al.*

### Conclusion

All grounds of rejection and objection of the pending Office Action having been addressed, reconsideration of the application is respectfully requested. It is respectfully submitted that the invention as claimed fully meets all requirements and that the claims are worthy of allowance. Should the Examiner believe that a telephone interview would aid in the prosecution of this application, Applicant encourages the Examiner to call the undersigned collect at (415) 705-8410.

Dated: April 18, 2000



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<sup>76</sup> Office Action, page 16, first full paragraph.

**APPENDIX I**  
**PENDING CLAIMS AS AMENDED IN THIS COMMUNICATION**

The following is a list of the claims as they would appear following entry of this amendment.

1. (Once amended) A method of producing a modification in a gene of interest contained in a cell, comprising:
  - a) providing:
    - i) an *in vitro* culture of target cells comprising isolated embryonic cells containing a gene of interest, said embryonic cells selected from embryonic stem cells, fertilized egg cells, and cells of 2-cell embryos;
    - ii) a chemical agent capable of producing at least one modification in said gene of interest in at least one of said embryonic cells and at least one modification in one or more additional genes;
  - b) treating said embryonic cells with said agent under conditions such that a mixture of embryonic cells is produced, said mixture of embryonic cells comprising cells having an unmodified gene of interest and cells having a modified gene of interest; and
  - c) isolating said cells having a modified gene of interest.
2. The method of Claim 1, further comprising step d) comparing the nucleotide sequence of said gene of interest in said cells having a modified gene of interest with the nucleotide sequence of said gene of interest in said cells having an unmodified gene of interest.
3. (Once amended) The method of Claim 1, further comprising d) placing at least one of said cells having a modified gene of interest into an environment under conditions so as to generate an organism comprising said modification in said gene of interest.
4. The method of Claim 2, further comprising prior to step d) amplifying said modified gene of interest to produce an amplified modified gene of interest.

5. The method of Claim 4, further comprising prior to step d) sequencing said amplified modified gene of interest.

6. The method of Claim 1, wherein said modification is selected from the group consisting of mutation, mismatch, and strand break.

7. The method of Claim 6, wherein said mutation is selected from the group consisting of deletion, insertion and substitution.

8. The method of Claim 6, wherein said strand break is selected from the group consisting of single-strand break and double-strand break.

9. (Once amended) The method of Claim 1, wherein said embryonic cells are derived from a non-human animal.

10. The method of Claim 9, wherein said non-human animal is a mammal.

11. The method of Claim 10, wherein said mammal is a mouse.

12. The method of Claim 9, wherein said non-human animal is zebrafish.

13. (Once amended) The method of Claim 1, wherein said embryonic cell is a mouse embryonic stem cell.

14. (Once amended) The method of Claim 1, wherein said agent is selected from the group consisting of *N*-ethyl-*N*-nitrosurea, methylnitrosourea, procarbazine hydrochloride, triethylene melamine, acrylamide monomer, chlorambucil, melphalan, cyclophosphamide, diethyl sulfate, ethyl methane sulfonate, methyl methane sulfonate, 6-mercaptopurine, mitomycin-C, procarbazine, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine,  $^3\text{H}_2\text{O}$ , and urethane.

15. (Once amended) A method of producing an allelic series of modifications in a gene of interest contained in a cell, comprising:

- a) providing:
  - i) an *in vitro* culture of target cells comprising isolated embryonic cells containing a gene of interest, said embryonic cells selected from embryonic stem cells, fertilized egg cells, and cells of 2-cell embryos;
  - ii) a chemical agent capable of producing at least one modification in said gene of interest in at least one of said embryonic cells;
- b) treating said embryonic cells with said agent under conditions such that a mixture of embryonic cells is produced, said mixture of embryonic cells comprising cells having an unmodified gene of interest, cells having a first modification in said gene of interest, and cells having a second modification in said gene of interest; and
- c) isolating said cells having a first modification in said gene of interest and said cells having a second modification in said gene of interest, thereby producing an allelic series of modifications in said gene of interest in the isolated cells.

16. The method of Claim 15, further comprising step d) comparing the nucleotide sequence of said gene of interest in said cells having an unmodified gene of interest with the nucleotide sequence of said gene of interest in cells selected from the group consisting of said cells having a first modification in said gene of interest and said cells having a second modification in said gene of interest.

17. (Once amended) The method of Claim 15, further comprising d) placing at least one cell selected from the group consisting of said cells having a first modification in said gene of interest and said cells having a second modification in said gene of interest into an environment under conditions so as to generate an organism comprising a modification selected from the group consisting of said first modification in said gene of interest and said second modification in said gene of interest.

18. The method of Claim 16, further comprising prior to step d) amplifying said gene of interest selected from the group consisting of said gene of interest having said first modification and said gene of interest having said second modification to produce amplified

modified gene of interest selected from the group consisting of amplified gene of interest having said first modification and amplified gene of interest having said second modification.

19. The method of Claim 18, further comprising prior to step d) sequencing said amplified modified gene of interest.

20. The method of Claim 15, wherein said first modification and said second modification are selected from the group consisting of mutation, mismatch, and strand break.

21. The method of Claim 20, wherein said mutation is selected from the group consisting of deletion, insertion and substitution.

22. The method of Claim 20, wherein said strand break is selected from the group consisting of single-strand break and double-strand break.

23. (Once amended) The method of Claim 15, wherein said embryonic cells are derived from a non-human animal.

24. The method of Claim 23, wherein said non-human animal is a mammal.

25. The method of Claim 24, wherein said mammal is a mouse.

26. The method of Claim 23, wherein said non-human animal is zebrafish.

27. (Once amended) The method of Claim 15, wherein said embryonic cell is a mouse embryonic stem cell.

28. (Once amended) The method of Claim 15, wherein said agent is selected from the group consisting of *N*-ethyl-*N*-nitrosurea, methylnitrosourea, procarbazine hydrochloride, triethylene melamine, acrylamide monomer, chlorambucil, melphalan, cyclophosphamide,

diethyl sulfate, ethyl methane sulfonate, methyl methane sulfonate, 6-mercaptopurine, mitomycin-C, procarbazine, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine,  $^3\text{H}_2\text{O}$ , and urethane.

29. (New) A method of producing a modification in a gene of interest contained in a cell, comprising:

- a) providing:
  - i) an *in vitro* culture of target cells comprising isolated embryonic cells containing a gene of interest, said embryonic cells selected from protocorm-like body cells, and callus cells.;
  - ii) a chemical agent capable of producing at least one modification in said gene of interest in at least one of said embryonic cells and at least one modification in one or more additional genes;
- b) treating said embryonic cells with said agent under conditions such that a mixture of embryonic cells is produced, said mixture of embryonic cells comprising cells having an unmodified gene of interest and cells having a modified gene of interest;
- c) isolating said cells having a modified gene of interest; and
- d) placing at least one of said cells having a modified gene of interest into an environment under conditions so as to generate an organism comprising said modification in said gene of interest.

30. (New) A method of producing a modification in a gene of interest contained in a mouse cell, comprising:

- a) providing:
  - i) an *in vitro* culture of isolated mouse embryonic stem cells containing a gene of interest;
  - ii) a chemical agent capable of producing at least one modification in said gene of interest in at least one of said mouse embryonic stem cells and at least one modification in one or more additional genes;
- b) treating said mouse embryonic stem cells with said agent under conditions such that a mixture of embryonic stem cells is produced, said mixture of

embryonic stem cells comprising cells having an unmodified gene of interest and cells having a modified gene of interest;

- c) isolating said cells having a modified gene of interest;
- d) comparing the nucleotide sequence of said gene of interest in said cells having a modified gene of interest with the nucleotide sequence of said gene of interest in said cells having an unmodified gene of interest; and
- e) manipulating said cells having a modified gene of interest to generate an organism comprising said modification in said gene of interest, wherein said manipulating comprises:
  - i) introducing said cells having said modified gene of interest into a mouse embryo to produce a treated embryo;
  - ii) introducing said treated embryo into a pseudopregnant mouse;  
and
  - iii) permitting said pseudopregnant mouse to deliver at least one offspring comprising said modified gene of interest.

31. (New) The method of Claim 3, wherein said organism is chimeric.

32. (New) A method of producing an allelic series of modifications in a gene of interest contained in a cell, comprising:

- a) providing:
  - i) an *in vitro* culture of target cells comprising isolated embryonic cells containing a gene of interest, said embryonic cells selected from protocorm-like body cells, and callus cells;
  - ii) a chemical agent capable of producing at least one modification in said gene of interest in at least one of said embryonic cells;
- b) treating said embryonic cells with said agent under conditions such that a mixture of embryonic cells is produced, said mixture of embryonic cells comprising cells having an unmodified gene of interest, cells having a first modification in said gene of interest, and cells having a second modification in said gene of interest;

- c) isolating said cells having a first modification in said gene of interest and said cells having a second modification in said gene of interest, thereby producing an allelic series of modifications in said gene of interest in the isolated cells; and
- d) placing at least one cell selected from the group consisting of said cells having a first modification in said gene of interest and said cells having a second modification in said gene of interest into an environment under conditions so as to generate an organism comprising a modification selected from the group consisting of said first modification in said gene of interest and said second modification in said gene of interest.

33. (New) A method of producing an allelic series of modifications in a gene of interest contained in a mouse cell, comprising:

- a) providing:
  - i) an *in vitro* culture of mouse embryonic stem celis;
  - ii) a chemical agent capable of producing at least one modification in said gene of interest in at least one of said mouse embryonic stem cells;
- b) treating said mouse embryonic stem cells with said agent under conditions such that a mixture of embryonic stem cells is produced, said mixture of embryonic stem cells comprising cells having an unmodified gene of interest, cells having a first modification in said gene of interest, and cells having a second modification in said gene of interest;
- c) isolating said cells having a first modification in said gene of interest and said cells having a second modification in said gene of interest, thereby producing an allelic series of modifications in said gene of interest in the isolated cells;
- d) comparing the nucleotide sequence of said gene of interest in said cells having an unmodified gene of interest with the nucleotide sequence of said gene of interest in cells selected from the group consisting of said cells having a first modification in said gene of interest and said cells having a second modification in said gene of interest; and
- e) manipulating cells selected from the group consisting of said cells having a first modification in said gene of interest and said cells having a second

modification in said gene of interest to generate an organism comprising a modification selected from the group consisting of said first modification in said gene of interest and said second modification in said gene of interest, wherein said manipulating comprises:

- i) introducing said cells having said first modification in said gene of interest and said cells having said second modification of said gene of interest into a mouse embryo to produce a treated embryo;
- ii) introducing said treated embryo into a pseudopregnant mouse;  
and
- iii) permitting said pseudopregnant mouse to deliver at least one offspring comprising said first modification in said gene of interest or said second modification in said gene of interest.

34. (New) The method of Claim 17, wherein said organism is chimeric.